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EVALUATION OF NITRIFICATION INHIBITORS IN COLD REGIONS LAND TREATMENT OF WASTEWATER: PART 1. NITRAPYRIN

S.M. Elgawhary, I.K. Iskandar and B.J. Blake

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A series of laboratory and field tests were conducted to investigate the possibility that nitrapyrin could be useful as a nitrification inhibitor in land treatment of wastewater. Laboratory tests included soil incubation and soil column studies. Variables were soil type, temperature, nitrapyrin concentration and method of application to the soil. Experimental designs included two soils, three temperatures (0°, 10° and 20°C) and three levels of inhibitors in a complete factorial. Forage grasses were present in all treatments, and wastewater containing NH_4^+ was utilized. Weekly application of wastewater was 5 cm. Soil solution at depth and leachate at 160 cm were collected and analyzed weekly for NH_4-N and NO_3-N.

That data indicate that nitrapyrin was not effective in inhibiting nitrification when applied to the soil surface in — cont

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soil columns simulating land treatment slow infiltration. The ineffectiveness of the compound under a mode of application where it is mixed and sprayed with wastewater is thought to be due to its volatility, sorption by organic matter, low water solubility and its immobility in soils. Other chemicals such as carbon disulfide and thiocarbonates, which have different characteristics than the nitrapyrin, showed promising results. Research is under way to obtain conclusive data.

Preface

This report was prepared by Dr. S. M. Elgawhary, Senior Research Scientist, Environmental Sciences (Rocky Flats, Energy Systems Group) Rockwell International Corporation, and by Dr. I. K. Iskandar, Research Chemist, and B. J. Blake, Physical Sciences Aid, Earth Sciences Branch, Research Division, U.S. Army Cold Regions Research and Engineering Laboratory. Dr. Elgawhary conducted this research during his visit to CRREL under Grant Agreement DACA 89-78-G-001.

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The authors would like to acknowledge the technical assistance of T. F. Jenkins, P. Schumacher and S. Quarry for sample analysis. This report was technically reviewed by Dr. D. R. Keeney, Department of Soil Science, University of Wisconsin, Dr. A. P. Edwards, Senior Research Scientist, University of New Hampshire, and Dr. C. Gray, Medical School, Dartmouth College.

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INTRODUCTION

The nitrogen loading rate appears to be critical in limiting the amount of wastewater that can be applied in land infiltration systems (Iskandar et al. 1976 Lee 1976). Domestic wastewaters normally contain 10 to 30 mg/l of N, largely as NH_4^+. The small fraction of soluble organic N which is usually present in wastewater is readily mineralized and converted to NH_4^+ by the microorganisms in the soil. The larger fraction of organic-N is likely to be associated with the solid fraction and will be slowly mineralized and converted to NH_4^+ by soil microorganisms. On contact with the soil, NH_4^+ will be largely sorbed on the soil exchange complex and further movement downward is limited. At this point NH_4^+ can either be taken up by plant roots or nitrified by the soil-nitrifying bacteria.

While nitrification proceeds rapidly in warm, well-aerated soils of neutral pH, it will also occur to some extent in cold soils. The resulting nitrate ions (NO_3^-), plus whatever originally was present in the wastewater can either be taken up by plant roots, denitrified (this would likely be significant only under warm, wet conditions) or leached to the groundwater. While the standard limit for NO_3-N in drinking water is set at 10 mg/l, it is generally accepted that excessive growth of aquatic plants and potential eutrophication can take place in surface water whenever NO_3-N exceeds a few tenths of a milligram per liter. Since groundwater frequently becomes, or contributes to, surface water, land infiltration systems must strive toward the removal of N from the percolate and the minimization of NO_3^- leached into the groundwater.

Enhanced denitrification is not technically feasible, especially in slow infiltration systems designed to be an integral part of an agricultural setting. This leaves uptake by plant roots as the only plausible route for removing significant amounts of N and minimizing leaching losses. However, N removal efficiencies are seasonally dependent (Iskandar et al. 1976), especially in climates with severe winters. Cold conditions retard nitrification, and NH_4^+ is retained during the winter months. In the spring, soils warm rapidly, and the rate of nitrification will far exceed the N uptake capacity of the vegetation, leading to high NO_3-N in the percolate. Even during the summer when growth is maximal, high NO_3-N concentrations are possible since water movement through the root zone would remove NO_3-N faster than it can be intercepted by the plant roots.

One possible approach to lowering NO_3-N in the leachate is to retard nitrification year-round through the use of a nitrification inhibitor. This would retain N in the root zone (as NH_4^+) and permit maximum uptake by plants. If a plant species that utilizes NH_4^+ equally as well as NO_3^- were grown, and if a management system were designed to add N to match plant needs, minimal NO_3-N leaching should result.

Work reported to date on the use of nitrification inhibitors in soils is exclusively related to the control of nitrification in ammonium or ammonium-yielding fertilizers as applied to agricultural land.

Nitrapyrin, 2-chloro-6-(trichloromethyl) pyridine, has been the nitrification inhibitor most widely used with NH_4+fertilizers and has been shown effective in controlling nitrification under such conditions. Nitrapyrin has low toxicity, degrades rapidly in the soil, and is available at relatively low cost. It has a very low water solubility, is readily sorbed by organic matter, and does not move significantly beyond the point of application. It is volatile and must be applied at least 2 to 4 in. below the soil surface to be effective (Goring 1962). An extensive up-to-date review on the factors affecting the persistence and bioactivity of nitrification inhibitors (mainly nitrapyrin) was recently presented by Keeney (1978). To our knowledge, no research on nitrapyrin or any other nitrification inhibitor in relation to wastewater land treatment has been conducted.

The objective of this research was to evaluate the effectiveness of nitrapyrin in inhibiting nitrification of wastewater NH_4-N in a slow infiltration land disposal system.

MATERIAL AND METHODS

Two New Hampshire soils were used in this study, Windsor sandy loam and Charlton silt loam. The Windsor soil had 2% clay and 27% silt while the Charlton soil had 13% clay and 33% silt. Both soils contained slightly more fine than medium sand and very little coarse sand. The Windsor soil had a pH of 6.0 and 6.3 and a cation exchange capacity (CEC) of 7.0 and 6.2 meq/100 g at the 0-to 15-cm and 5-to 30-cm depths, respectively, while the Charlton soil had a pH of 6.6 and 6.4 and a CEC of 13.5 and 8.2 meq/100g at the same respective depths.

Soil column study

Eighteen PVC columns, each 60 cm deep and 20 cm in diameter (Fig. 1), were packed with either Windsor sandy loam or Charlton silt loam soils (9 each) and sodded with orchardgrass. Three columns of each soil were maintained under three different temperature regimes, 0°, 10°, and 27°C. Primary wastewater (25-30 ppm NH_4-N and zero NO_3-N) was applied to the columns at the rate of 5 cm/week. Columns kept at 27°C received 7.5 cm of wastewater/week for three weeks prior to the harvest of the grass. Replicate columns at each temperature received nitrapyrin mixed with the wastewater at the rate of 4%, 2% or 0% by weight of the inhabator relative to the weight of the NH_4-N in the weekly wastewater addition. For two weeks prior to collection of the data presented here, wastewater treated with nitrapyrin according to the experimental design and tapwater were added alternately to all the columns in 5-cm weekly additions to leach out any nitrate originally present in the soil. Soil solution samples were collected weekly under 60-centibar suction from each column at depths of 16, 36, 56 cm and were analyzed for NO_3^- and NH_4^+ content using a Technicon II Autoanalyzer with the cadmium reduction method for NO_3^- and an adaptation of the Kjeldahl total N method for NH_4^+. The grass was harvested periodically and yield and N content were determined.

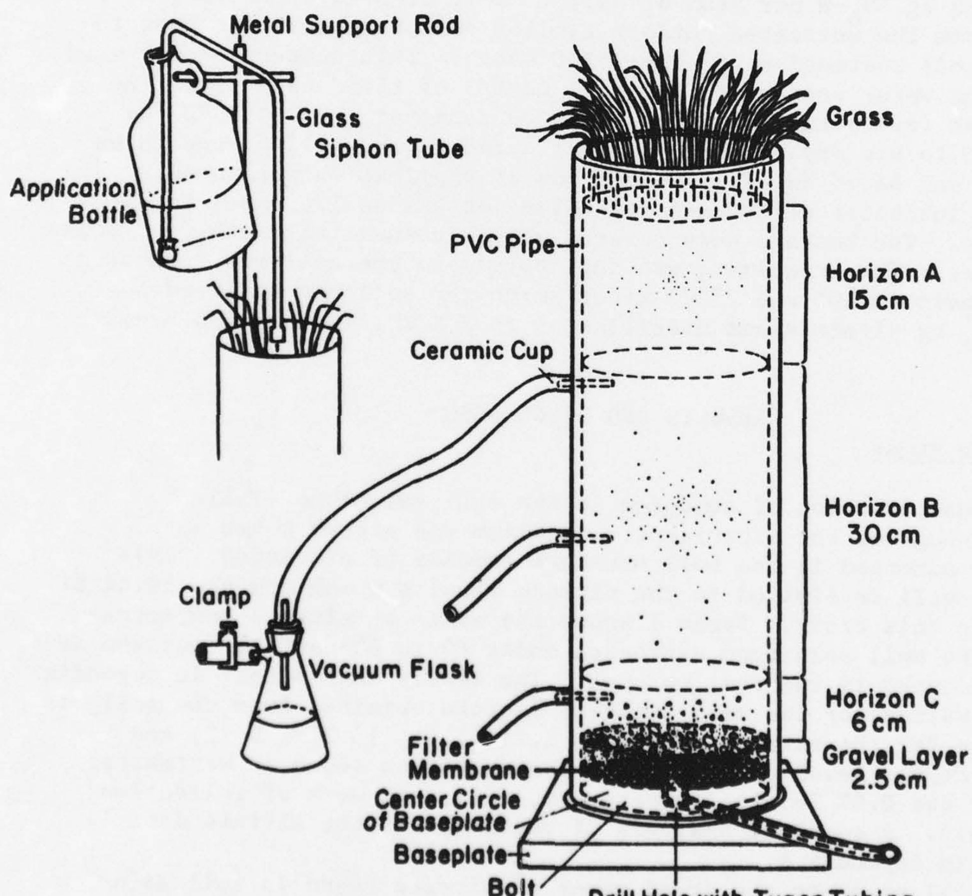

Figure 1. Soil columns and application method.

Outdoor test cell study

Two outdoor large-scale (8.5- x 8.5-m) test cells at the CRREL Land Treatment Research Facility in Hanover, New Hampshire, were used in this study. One cell contained Windsor sandy loam soil while the other contained Charlton silt loam. The cells received 5 cm of primary wastewater each week mixed with nitrapyrin at the rate of 2% of the NH_4-N present. Percolate at the bottom of the cell was collected twice a week and analyzed for NO_3^- and NH_4^+. In October 1978, soil samples (0-7.5 cm) were collected daily for seven consecutive days from nitrapyrin-treated and untreated test cells and analyzed for NH_4^+ and NO_3-N. Grass was harvested periodically.

Soil incubation experiment

Windsor sandy loam soil was used in the soil incubation experiment. Samples (100 g) in 150-ml glass beakers were treated with $NH_4H_2PO_4$ solution (60 µg NH_4-N per gram of soil). Soil rich in nitrifying bacteria from the untreated outdoor Windsor soil test cell was used to prepare a soil suspension of 5 soil:100 water. This suspension was used to bring the water content of the soil to 2/3 of field capacity. The samples were incubated for 18 days at room temperature (20° to 22°C) and allowed to air dry. Nitrapyrin was added to the soil at the rates of 0, 2, 4 and 6% of NH_4-N. Two methods of application were used, mixing the inhibitor with the total volume of the soil or applying it to the surface. The beakers were covered with aluminum foil with four holes in the cover. The experiment was duplicated and the soil was incubated for five weeks at 10° and 27°C, after which the soil was analyzed for NO_3^- and NH_4^+ by direct steam distillation in 2 M KCl (Keeney and Bremner 1966).

RESULTS AND DISCUSSION

Soil column study

The concentration of ammonium in the soil solutions (Table I) was low throughout the experiment. Ammonium was either taken up by the grass, adsorbed in the soil exchange complex or nitrified. This discussion will be limited to the nitrate results which are the critical criteria in this study. Table I shows the means of nitrate concentrations in the soil solutions extracted under 60-to 65-centibar suction at different depths in the soil columns. The weekly data appear in Appendix A. The F values for the main (single) factors obtained from the analysis of variance for the nitrate data were 1.21, 1.44, 15.37*, 0.12, and 2.93† for depth, level of nitrapyrin concentration added to wastewater (including the 0.0% level), temperature, soil, and week of collection, respectively. A complete analysis of variance for the nitrate data is presented in Appendix A.

Table II presents the grand means of nitrate found in soil solutions, calculated for each level of the factors in the experimental design over the range of the whole experiment.

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* Value highly significant to the study.

† Value significant to the study.

Table I. Average values for nitrate-N and ammonium-N in soil solution (collected weekly) over the period of seven weeks.

Windsor			Crarlton			Treatments	
Col. no. and depth	NO_3 (ppm)	NH_4 (ppm)	Col. no. and depth	NO_3-N (ppm)	NH_4-N (ppm)	Temp. (°C)	Nitrapyrin % of NH_4-N
1-A†	0.7	2.7	10-A	0.9	2.7	0	0.0
B	2.1	1.3	B	0.1	1.4		
C	1.5	1.0	C	18.5	0.9		
2-A	1.8	0.1	11-A	0.3	4.9	0	2.0
B	2.9	0.01	B	0.04	1.1		
C	2.4	0.2	C	0.04	1.1		
3-A	0.9	3.4	12-A	0.003	4.4	0	4.0
B	1.1	1.6	B	0.01	1.7		
C	0.5	1.3	C	0.4	1.4		
4-A	3.0	2.1	13-A	0.4	3.9	10	0.0
B	3.2	1.8	B	0.01	1.9		
C	2.3	1.5	C	0.04	1.5		
5-A	0.4	3.2	14-A	0.06	1.9	10	2.0
B	0.9	1.8	B	0.01	1.9		
C	0.6	1.9	C	0.3	1.7		
6-A	0.9	3.7	15-A	1.1	0.3	10	4.0
B	0.03	1.6	B	0.01	1.8		
C	0.03	1.7	C	0.02	1.5		
7-A	31.3	0.03	16-A	4.2	0.2	27	0.0
B	19.3	0.02	B	46.2	0.9		
C	19.5	0.04	C	47.0	0.6		
8-A	19.1	0.01	17-A	0.6	0.6	27	2.0
B	9.5	0.05	B	7.1	1.1		
C	18.7	0.05	C	9.6	0.9		
9-A	2.0	0.04	18-A	4.8	0.5	27	4.0
B	8.3	0.01	B	57.5	1.0		
C	45.9	0.07	C	37.0	0.6		

$LSD_{0.05}$ = 9.5 (for NH_4), $LSD_{0.01}$ = 12.7 (for NO_3).

†A, B, and C represent depths of 16, 36, and 56 cm from column surface.

Table II. Grand means of nitrate (ppm) in soil solution calculated for each level of the factors involved over the range of the whole experiment.

Level of factor	Factor				
	Depth*	Nitrapyrin concentration†	Temp.**	Soil type	Week††
1	4.0	11.1	1.9	7.4	5.5
2	8.8	4.1	0.7	8.7	4.4
3	11.3	8.9	21.5		6.0
4					8.6
5					10.1
6					11.4
7					10.4
$LSD_{0.05}$	13.3	11.8	11.7	10.8	5.3
$LSD_{0.01}$			19.4		7.2

* Depth 1, 2, and 3 = 16, 36, and 58 cm, respectively.

† Nitrapyrin concentration 1, 2, and 3 = 0.0, 2.0, and 4.0%, respectively, of the NH_4-N in applied wastewater.

**Temperature 1, 2, and 3 = 0°C, 10°C, and 27°C, respectively.

††Week 1, 2, ..., and 7 = first, second, ... seventh collection week.

From Tables I and II and the F values of the analysis of variance, it appears clear that: 1) temperature was the only factor that significantly controlled and affected the process of nitrification under the system studied here, 2) the variance in the nitrate content of the soil solution was due in the first place to the temperature effect and to a much lesser extent to the cumulative effect of the weekly wastewater application and 3) nitrapyrin under this mode of application (i.e. mixed with wastewater and applied to the surface of the soil) had no significant effect in inhibiting or controlling the process of nitrification and the transformation of ammonium to nitrate in wastewaters. However, there is a tendency (although not statistically significant) for a lower nitrate-N level in soil solution samples collected from the upper two depths of the soil columns treated with nitrapyrin than from those collected from the untreated columns. A statistically significant double interaction among the factors included in the experimental design was the interaction of temperature and week of collection ($F = 4.96$).

Table III shows the means of nitrate in soil solution for this interaction. Values in the table indicate that the nitrate-N concentration in the soil solution in columns maintained at 0° and 10°C was very low and more or less constant. However, nitrate-N concentration in the 27°C columns increased appreciably with time before it leveled off. This initial increase in nitrate-N concentration reflected an cumulative effect of the nitrifiable wastewater-NH_4^+ applied weekly. The leveling off probably involved a question of equilibrium of inputs and nitrification with plant uptake of NO_3-N.

Table III. Mean of nitrate (ppm) in soil solution for the two soils, three depths, and three levels of nitrapyrin concentration.

Week	Temperature (°C) 0	10	27
1	6.4	0.7	9.4
2	2.7	0.9	9.6
3	1.2	0.8	15.9
4	0.9	0.5	24.5
5	0.8	0.5	29.2
6	0.8	0.7	32.7
7	0.7	1.0	29.4

$LSD_{0.05} = 10$

$LSD_{0.01} = 13.5$

The ineffectiveness of nitrapyrin, when mixed with wastewater and applied to the soil surface, in inhibiting the nitrification of wastewater-NH_4^+ is probably due to: 1) sorption by organic matter, 2) low water solubility, 3) volatility, and 4) degradation in soils. Nitrapyrin is highly sorbed by the soil organic matter; its distribution ratio of sorption by the soil organic matter to that in water ranged from 86:1 to 262:1 with a mean of 151:1 (Goring 1962a). Evaluation studies showed a decrease in its effectiveness and bioactivity as organic matter increased (Goring 1962a and b, Bundy and Bremner 1973, Geronimo et al. 1973, Lewis and Stefanson 1975, Hendrikson et al. 1978a), and organic-matter-sorbed nitrapyrin was found not to affect nitrification (Laskowski and Bidlack 1977). Thus, the thick grass root-mat developed through the upper 4 or 5 cm of the soil columns and the extensive root system in the upper 20 or 25 cm may very well account for the sorption of most of the nitrapyrin applied to the soil surface and for a significant decrease in its effectiveness in the upper portions of the treated columns.

The high affinity to organic matter and the low water solubility of nitrapyrin resulted in its low mobility in the system. At the same time, ammonium moved more rapidly down the column through the processes of diffusion and mass flow. This differential movement of ammonium and nitrapyrin resulted in inhibitor-free zones at the lower portions of the soil columns and thus little or no nitrification of NH_4^+ that moved down the columns. The overall effect of this affinity to organic matter and low mobility of nitropyrin was a localized and very limited (not statistically significant) nitrification control at the upper portions of the soil columns. This was evidenced by a tendency to lower nitrate levels in soil solutions extracted from the upper portions of the nitrapyrin-treated columns. Furthermore, the ineffectiveness of the compound may have been accentuated by its loss from the surface through volatilization and by its degradation in the soil to 6-chloropicolinic acid (6-CPA) which has limited bioactivity. Both volatility and degradation of nitrapyrin are greatly increased with increasing temperature (Goring 1962a and b, Touchton et al. 1978, Hendrickson et al. 1978b).

In the absence of nitrification control by nitrapyrin, the temperature effect on the enhancement of wastewater-NH_4 nitrification is expected. The optimum temperature for nitrification in soils is known to be between 27° and 33°C; it stops completely at freezing and is slow at 6° to 7°C. These are temperatures which coincide closely with the range of temperatures used in the study. The results obtained here (Table I) show very little NO_3^- in soil solutions at both 0° and 10°C. No plant growth took place at 0°C and it seems that no nitrification occurred. Also, there was little plant growth on the 10°C columns. The small amount of nitrate which may have resulted from the slow process of nitrification at 10°C was probably taken up by the plants. Plant uptake for the 27°C columns accounted for 78, 80, and 74% of the N applied to the 0%, 2% and 4% nitrapyrin-treated Windsor soil columns, respectively.

In the Charlton soil (at 27°C), uptake by plants accounted for 51, 56, and 68% of the N added to the 0%, 2% and 4% nitrapyrin-treated columns, respectively.

Outdoor test cells

Nitrate concentrations in the leachate collected from the nitrapyrin-treated test cells (data not presented) did not differ significantly from those of the untreated cells. Again, the ineffectiveness of nitrapyrin in inhibiting nitrification in this system is probably due to organic matter, immobility, and degradation in the soil.

Table IV shows the NH_4-N and NO_3-N values in soil samples taken daily for seven days (starting on 24 October 1978) from nitrapyrin-treated test cells (cells 3 and 4). Statistical analysis showed that there is a significant difference between the nitrapyrin-treated and untreated soils (significant at the 5% level) in their NH_4-N content. The effects of soil type and the number of days after treatment with wastewater and nitrapyrin were not significant. In contrast there was no significant difference between the treated and untreated cells due to any of the three factors; nitrapyrin, soils or days after treatment. This is not surprising since NO_3^- is known to be leachable and/or taken up by plants. Table V summarizes the analysis of variance for both NH_4^+ and NO_3^-.

Soil incubation experiment

Table VI shows the means of the NO_3-N and the NH_4-N concentrations of the soil after incubation. Each value represents the average of the analyses of three subsamples of the moist soil from each of the duplicate beakers. A check treatment that did not receive any NH_4^+ had 0.48 mg NH_4-N and 0.81 mg NO_3-N per 100 g soil incubated at 10°C, and 0.11 mg NH_4-N and 1.0 mg NO_3-N per 100 g soil at 27°C.

Results were inconclusive with regard to the effect of the inhibitors used to control nitrification. At 10°C, the effect of low temperature in controlling nitrification overshadowed other factors. The NH_4^+ added to the soil stayed at about the same level at the end of the 10°C incubation; very little nitrification took place, especially if the NH_4^+ and NO_3^- values of the check soil are considered. The nitrate concentrations in the soil incubated at 27°C were approximately the same as those in the 10°C soil. However, there was a drastic decrease in the NH_4^+ content of the 27°C incubated soil, indicating a net loss of N (soluble and exchangeable) from the soil that was most probably in the NH_4^+ form. This major decrease in N concentration could be explained by ammonium fixation, denitrification, N immobilization, or volatilization of NH_3 in association with water loss under the relatively high temperature incubation conditions. Ammonium fixation should have occurred at the same rate under the two different temperatures. However, the loss occurred only at one temperature.

To assume denitrification, nitrification had to first take place. Although the results here (Table IV) are inconclusive regarding nitrification and its inhibition, the inhibitors used in the study are known

Table IV. Concentration (meq/100 g soil) of NH_4-N and NO_3-N in soils (0–7.5 cm) treated or untreated with nitrapyrin.

Days after treatment	Test cell*	NH_4-N (a)+	NH_4-N (b)	NO_3-N (a)	NO_3-N (b)
		meq/100g			
	2	0.099	0.094	0.025	0.021
1	3	0.032	0.027	0.017	0.024
	4	0.041	0.033	0.034	0.038
	5	0.025	0.039	0.018	0.029
	2	0.014	0.017	0.010	0.009
2	3	0.020	0.023	0.018	0.020
	4	0.061	0.062	0.034	0.028
	5	0.021	0.023	0.014	0.015
3	2	0.447	0.481	0.055	0.061
3	3	0.040	0.013		0.021
	4	0.057	0.090	0.041	0.058
	5	0.069	0.068	0.021	0.022
	2	0.114	0.115	0.031	0.031
4	3	0.014	0.026	0.028	0.024
	4	0.041	0.048	0.031	0.030
	5	0.055	0.060	0.035	0.029
	2	0.109	0.110	0.017	0.020
5	3	0.023	0.027	0.025	0.025
	4	0.018	0.027	0.018	0.030
	5	0.080	0.088	0.019	0.031
	2	0.070	0.288	0.044	0.043
6	3	0.037	0.024	0.018	0.016
	4	0.025	0.034	0.021	0.037
	5	0.321	0.372	0.032	0.045
	2	0.139	0.147	0.034	0.024
7	3	0.004	0.008	0.011	0.027
	4	0.002	0.018	0.013	0.027
	5	0.096	0.091	0.031	0.037

\+ Soil core (a) or (b)

* Test cells 2 and 5 were treated with nitripyrin while 3 and 4 were not.

Table V. Analysis of variance for NH_4^+ and NO_3^- concentrations in soils treated or untreated with nitrapyrin.

Factor*	Sum of SQ	DF	Mean SQ	F value
			Ammonium Concentration	
S	0.0063	1	0.0063	0.382
N	0.1378	1	0.1378	8.31†
T	0.1206	6	0.0201	1.21
SN	0.0208	1	0.0208	1.26
ST	0.0729	6	0.0121	0.733
NT	0.1078	6	0.0179	1.08
SNT	0.0994	6	0.0166	16.99**
Within-cell error	0.0273	28	0.0009	
			Nitrate Concentration	
S	2.516×10^{-4}	1	2.516×10^{-4}	0.647
N	1.275×10^{-4}	1	1.275×10^{-4}	0.328
T	1.467×10^{-3}	6	2.445×10^{-4}	0.629
SN	8.033×10^{-4}	1	8.033×10^{-4}	2.066
ST	8.773×10^{-5}	6	1.462×10^{-5}	0.038
NT	1.452×10^{-3}	6	2.419×10^{-4}	0.622
SNT	2.333×10^{-3}	6	3.888×10^{-4}	9.16**
Within-cell error	1.188×10^{-3}	28	4.24×10^{-5}	

† Significant at 5% level

* S means soil type, S1 is Windsor soil, S2 is Charlton soil; N means treatment with Nitrapyrin; T means time in days from application of wastewater or wastewater and inhibitor, T1 is one day, T2 is two days, etc.

** Significant at 1% level

Table VI. Averages of nitrate and ammonium contents of the soil after incubation.

Inhibitor*	NH_4-N (%)	Appl. method	10°C NO_3-N	10°C NH_4-N	27°C NO_3-N	27°C NH_4-N
			mg/100 g soil			
T	0	M	1.3	5.11	0.91	0.11
T	0	S	1.3	5.03	1.0	0.10
T	2	M	1.8	6.11	1.0	0.52
T	2	S	1.8	6.40	0.8	0.18
T	4	M	1.8	6.7	1.0	0.27
T	4	S	1.7	6.35	1.6	0.13
T	6	M	1.6	6.85	1.0	0.37
T	6	S	1.6	7.48	1.3	0.17
N	0	M	1.3	5.11	0.9	0.11
N	0	S	1.4	5.03	1.0	0.10
N	2	M	1.2	6.2	0.9	0.11
N	2	S	1.3	5.3	1.4	0.94
N	4	M	1.2	5.9	1.1	0.16
N	4	S	1.7	6.6	1.3	0.27
N	6	M	1.1	6.0	1.5	1.64
N	6	S	1.7	6.6	1.6	3.22
LSD 0.05			0.58	0.77	0.58	0.38

T = sodium trithiocarbonate
N = nitrapyrin
M = mixed application
S = surface application

to exert some inhibitory effect on nitrification in incubation studies. This will somewhat tend to eliminate denitrification as the major pathway for N loss from the system. Some of the soluble N could have been immobilized, but it seems that the loss of ammonia in association with water evaporation from the system could primarily explain the major loss of N. Several investigators have observed the association between water evaporation and NH_3 loss from the soil and that NH_3 in the soil is not emitted in significant quantities until water is evaporated (Allison 1966 Lauer et al. 1976 Denmead et al. 1974 and 1978). In this study soil kept under 27°C lost water rapidly, and water had to be replenished frequently to conserve the moisture level in the soil by bringing the beakers to a constant weight.

CONCLUSIONS

Nitrapyrin was not effective in inhibiting nitrification when applied to the soil surface in a system simulating land treatment of wastewater by slow infiltration. The ineffectiveness of the compound under a mode of application where it is mixed and sprayed with wastewater is due to its volatility, sorption by organic matter, low water solubility and its immobility in soils.

Unless modes of soil subsurface application for nitrapyrin are developed and proved to be practicable to use in the land treatment of wastewater, the compound has no potential use in such systems.

FUTURE RESEARCH IDEAS

The inhibitory effect of carbon disulfide on nitrification in soils was first recognized by Powlson and Jenkinson (1971) when soils incubated in all-glass respirometers were compared with those incubated in rubber-stoppered respirometers. They identified the CS_2 released from the rubber stoppers as the cause for inhibiting nitrification in the soil. Ashworth et al. (1975) found that nitrification inhibition by CS_2 was superior to that by nitrapyrin, mainly because CS_2 diffused through the fertilizer band while nitrapyrin remained close to the injection point. Kudeyarov and Jenkinson (1976) reported that CS_2 at low concentrations (10 μg/g soil) can inhibit nitrification while not affecting soil respiration or mineralization of N. However, in a soil low in organic matter (1.07 %), that same low concentration of CS_2 was sufficient to increase both respiration and mineralization.

Carbon disulfide is not practicable to use, since it is volatile and flammable. Ashworth et al. (1977) evaluated sodium trithiocarbonate, Na_2CS_3, which is stable in alkaline aqueous solution, water soluble, nonvolatile and nonflammable. They found that Na_2CS_3 decomposed chemically to CS_2 within 24 hours in the soil. It diffused rapidly through the soil and inhibited nitrification in a larger volume of soil than did nitrapyrin, but had less residual effect.

The high mobility and diffusivity, water solubility, and nonvolatility which characterize the reactions of Na_2CS_3 in soil much favor it as a potentially highly effective and practicable nitrification inhibitor with wastewater in slow infiltration land treatment systems. An investigation of Na_2CS_3 effectiveness along the same lines used with nitrapyrin in this study is strongly recommended.

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APPENDIX A: WEEKLY DATA AND ANALYSIS OF VARIANCE

Nitrate-N and ammonium-N in soil solution were extracted from nitrapyrin-treated soil columns at three depths from the surface of the columns.

Depth A	16 cm from column surface
Depth B	36 cm from column surface
Depth C	58 cm from column surface

Columns 1, 4, 7, 10, 13, and 16 received nitrapyrin = 0.0%,
Columns 2, 5, 8, 11, 14, and 17 received nitrapyrin = 2.0%,
Columns 3, 6, 9, 12, 15, and 18 received nitrapyrin = 4.0%
of the NH_4-N in the applied wastewater.

Columns 1, 2, 3, 10, 11, 12 were kept at 0°C
Columns 4, 5, 6, 13, 14, 15 were kept at 10°C
Columns 7, 8, 9, 16, 17, 18 were kept at 27°C

Wastewater applied to 1 and 2 refer to two different samples of the wastewater applied weekly.

Table A-1

Collection Date: 18 Sept. 1978

Windsor			Charlton		
Col. No. and Depth	NO_3 ppm	NH_4 ppm	Col. No. and Depth	NO_3 ppm	NH_4 ppm
1-A	1.29	1.84	10-A	0.98	2.38
B	1.77	0.84	B	0.27	1.33
C	1.24	0.84	C	97.56	1.13
2-A	2.22	0.07	11-A	0.35	3.95
B	2.04	0.00	B	0.00	1.30
C	1.44	0.11	C	0.02	0.85
3-A	1.38	2.55	12-A	0.01	3.78
B	1.05	1.06	B	0.00	1.63
C	0.25	1.11	C	2.58	1.16
4-A	2.16	1.82	13-A	1.37	3.96
B	2.18	1.58	B	0.00	1.53
C	0.50	1.21	C	0.22	1.58
5-A	0.40	2.44	14-A	0.10	1.38
B	1.18	1.38	B	0.00	1.94
C	0.34	1.80	C	1.76	1.61
6-A	1.03	3.37	15-A	1.76	0.60
B	0.04	1.43	B	0.00	1.72
C	0.09	1.65	C	0.07	2.49
7-A	1.86	0.11	16-A	0.84	0.88
B	8.14	0.04	B	0.00	1.95
C	9.02	0.00	C	26.81	0.88
8-A	1.08	0.00	17-A	0.27	1.90
B	3.84	0.04	B	0.02	2.96
C	6.05	0.13	C	31.02	1.50
9-A	0.64	0.00	18-A	0.16	2.19
B	5.17	0.07	B	1.93	2.73
C	30.69	0.28	C	42.12	1.66
		Wastewater Applied	1	0.00	29.54
		15 Sept.	2	0.00	29.92

Table A-2

Collection Date: 25 Sept. 1978

Windsor			Charlton		
Col. No. and Depth	NO_3 ppm	NH_4 ppm	Col. No. and Depth	NO_3 ppm	NH_4 ppm
1-A	1.1	1.7	10-A	1.2	2.1
B	3.5	1.8	B	0.0	1.2
C	1.6	0.9	C	28.6	1.0
2-A	3.2	0.0	11-A	0.4	3.8
B	3.4	0.0	B	0.0	1.2
C	2.1	0.2	C	0.04	0.9
3-A	1.1	2.4	12-A	0.01	3.8
B	1.5	1.0	B	0.0	1.4
C	0.5	1.2	C	0.05	1.3
4-A	2.8	1.6	13-A	1.4	3.7
B	2.7	1.6	B	0.0	1.5
C	0.9	1.3	C	0.05	1.5
5-A	0.3	2.2	14-A	0.3	1.2
B	1.2	1.3	B	0.0	1.6
C	0.2	1.7	C	0.05	1.7
6-A	3.8	3.0	15-A	1.3	0.2
B	0.2	1.4	B	0.0	1.6
C	0.05	1.8	C	0.05	1.1
7-A	3.9	0.0	16-A	6.5	0.1
B	10.1	0.04	B	10.32	2.07
C	9.3	0.08	C	22.2	0.1
8-A	2.6	0.0	17-A	0.1	0.9
B	4.2	0.0	B	0.8	2.1
C	8.1	0.2	C	10.7	0.7
9-A	2.0	0.0	18-A	0.4	1.0
B	13.9	0.0	B	4.7	2.4
C	37.6	0.2	C	24.8	1.4
		Wastewater applied	1	0.00	33.76
		22 Sept	2	0.00	29.68

Table A-3

Collection Date: 2 Oct 1978

Windsor Col. No. and Depth	Windsor NO_3 ppm	Windsor NH_4 ppm	Charlton Col. No. and Depth	Charlton NO_3 ppm	Charlton NH_4 ppm
1-A	1.1	2.2	10-A	1.1	3.3
B	3.3	1.3	B	0.0	1.4
C	2.0	1.0	C	2.8	0.7
2-A	1.6	0.0	11-A	0.3	4.3
B	3.3	0.0	B	0.0	1.5
C	2.5	0.1	C	0.0	1.0
3-A	0.8	2.9	12-A	0.0	4.2
B	1.2	1.4	B	0.0	1.6
C	0.9	1.5	C	0.0	1.5
4-A	2.1	1.7	13-A	0.0	3.8
B	3.5	1.8	B	0.0	1.8
C	2.0	1.7	C	0.0	1.5
5-A	0.3	2.7	14-A	0.0	1.4
B	1.0	1.4	B	0.0	1.8
C	0.2	2.5	C	0.0	1.5
6-A	1.2	3.8	15-A	0.9	0.1
B	0.0	1.3	B	0.0	1.9
C	0.0	2.4	C	0.0	1.2
7-A	2.7	0.0	16-A	6.3	0.0
B	12.5	0.0	B	40.1	0.4
C	16.7	0.0	C	35.0	0.0
8-A	1.7	0.0	17-A	0.7	0.7
B	2.6	0.2	B	5.3	0.9
C	18.7	0.0	C	5.2	0.1
9-A	1.1	0.0	18-A	9.9	0.0
B	13.2	0.0	B	38.2	1.4
C	53.6	0.0	C	23.5	0.1
		Wastewater applied	1	0.1	25.3
		29 Sept	2	0.1	27.3

Table A-4

Collection Date: 10 Oct 1978

Windsor			Charlton		
Col. No. and Depth	NO_3 ppm	NH_4 ppm	Col. No. and Depth	NO_3 ppm	NH_4 ppm
1-A	0.6	2.7	10-A	1.0	2.4
B	2.1	1.2	B	0.2	1.5
C	1.7	1.1	C	0.1	0.7
2-A	0.7	0.0	11-A	0.2	4.4
B	3.6	0.0	B	0.2	1.6
C	2.9	0.2	C	0.0	1.1
3-A	0.8	3.5	12-A	0.0	4.3
B	1.2	1.5	B	0.1	1.7
C	0.5	1.4	C	0.0	1.4
4-A	1.7	1.8	13-A	0.0	3.9
B	3.5	1.8	B	0.1	1.9
C	2.4	1.5	C	0.0	1.5
5-A	0.4	2.8	14-A	0.0	1.6
B	0.7	1.2	B	0.1	2.1
C	0.0	1.9	C	0.0	1.6
6-A	0.0	3.4	15-A	0.2	0.2
B	0.0	1.5	B	0.1	1.9
C	0.0	1.7	C	0.0	1.5
7-A	27.1	0.0	16-A	4.5	0.0
B	15.7	0.0	B	67.9	0.1
C	21.7	0.1	C	45.0	0.2
8-A	12.0	0.0	17-A	1.5	0.1
B	4.7	0.0	B	5.7	0.9
C	22.5	0.0	C	1.0	0.7
9-A	4.2	0.0	18-A	9.9	0.0
B	10.1	0.0	B	91.6	0.1
C	56.8	0.0	C	38.9	0.2
		Wastewater applied	1	0.2	24.1
		6 Oct	2	0.0	24.4

Table A-5

Collection Date: 16 Oct 1978

Windsor			Charlton		
Col. No. and Depth	NO_3 ppm	NH_4 ppm	Col. No. and Depth	NO_3 ppm	NH_4 ppm
1-A	0.4	3.3	10-A	0.8	2.9
B	1.6	1.3	B	0.0	1.5
C	1.3	1.2	C	0.0	0.9
2-A	1.6	0.2	11-A	0.2	5.4
B	2.5	0.1	B	0.0	1.7
C	3.0	0.1	C	0.0	1.2
3-A	0.8	3.8	12-A	0.0	4.6
B	0.8	1.8	B	0.0	1.8
C	0.5	1.4	C	0.0	1.4
4-A	3.0	2.2	13-A	0.0	3.8
B	1.9	1.8	B	0.0	2.1
C	2.2	1.5	C	0.0	1.5
5-A	0.1	3.4	14-A	0.0	2.1
B	0.6	1.3	B	0.0	2.0
C	0.2	1.9	C	0.0	1.8
6-A	0.0	3.9	15-A	0.9	0.2
B	0.0	1.7	B	0.0	2.0
C	0.1	1.7	C	0.0	1.5
7-A	58.3	0.1	16-A	0.4	0.1
B	22.3	0.1	B	71.3	0.1
C	22.7	0.1	C	46.0	0.1
8-A	51.1	0.1	17-A	0.4	0.2
B	13.7	0.1	B	3.0	0.9
C	23.0	0.0	C	0.0	0.3
9-A	4.3	0.2	18-A	10.4	0.0
B	5.6	0.0	B	94.2	0.1
C	48.1	0.0	C	50.2	0.0
		Wastewater applied	1	0.0	28.9
		13 Oct	2	0.0	29.1

Table A-6

Collection Date: 23 Oct 1978

Windsor			Charlton		
Col. No. and Depth	NO_3 ppm	NH_4 ppm	Col. No. and Depth	NO_3 ppm	NH_4 ppm
1-A	0.6	2.5	10-A	0.6	2.8
B	1.2	1.4	B	0.1	1.4
C	1.5	1.2	C	0.0	0.8
2-A	2.1	0.2	11-A	0.2	6.1
B	3.0	0.0	B	0.1	1.8
C	2.3	0.1	C	0.2	1.2
3-A	1.0	4.1	12-A	0.0	5.2
B	0.9	2.0	B	0.0	1.9
C	0.5	1.4	C	0.2	1.4
4-A	2.9	2.7	13-A	0.0	4.0
B	3.6	2.0	B	0.0	2.1
C	2.7	1.5	C	0.0	1.4
5-A	0.6	4.1	14-A	0.0	2.5
B	0.6	5.0	B	0.0	2.1
C	0.2	1.8	C	0.0	1.7
6-A	0.3	4.0	15-A	1.0	0.5
B	0.0	1.8	B	0.0	1.7
C	0.0	1.5	C	0.0	1.4
7-A	57.2	0.0	16-A	9.0	0.5
B	26.8	0.0	B	69.0	1.6
C	27.4	0.0	C	80.3	2.6
8-A	49.1	0.0	17-A	1.3	0.0
B	13.4	0.0	B	18.2	0.0
C	26.9	0.0	C	10.1	3.0
9-A	1.4	0.1	18-A	0.1	0.4
B	8.0	0.0	B	91.8	0.0
C	49.1	0.0	C	50.0	0.0
		Wastewater applied	1	0.0	32.1
		20 Oct	2	0.0	31.7

Table A-7

Collection Date: 30 Oct 1978

Windsor			Charlton		
Col. No. and Depth	NO_3 ppm	NH_4 ppm	Col. No. and Depth	NO_3 ppm	NH_4 ppm
1-A	0.5	4.7	10-A	0.5	2.8
B	1.3	1.4	B	0.1	1.6
C	1.2	1.1	C	0.1	0.8
2-A	1.2	0.4	11-A	0.2	6.2
B	2.8	0.0	B	0.0	1.8
C	2.3	0.4	C	0.0	1.3
3-A	0.5	4.3	12-A	0.0	5.0
B	1.1	2.2	B	0.0	1.9
C	0.6	1.4	C	0.0	1.4
4-A	6.1	3.2	13-A	0.0	4.1
B	4.7	2.0	B	0.0	2.3
C	5.2	1.5	C	0.0	1.6
5-A	0.5	4.6	14-A	0.0	2.8
B	0.8	1.3	B	0.0	2.1
C	0.2	1.6	C	0.0	1.7
6-A	0.0	4.7	15-A	1.9	0.6
B	0.0	1.9	B	0.0	1.9
C	0.0	1.4	C	0.0	1.4
7-A	68.3	0.0	16-A	1.7	0.0
B	39.9	0.0	B	65.0	0.0
C	29.9	0.0	C	73.7	0.1
8-A	16.1	0.0	17-A	0.2	0.0
B	23.8	0.0	B	17.0	0.2
C	25.6	0.0	C	9.0	0.2
9-A	0.2	0.0	18-A	2.6	0.1
B	1.8	0.0	B	80.0	0.0
C	45.6	0.0	C	29.4	0.6
		Wastewater applied	1	0.2	31.4
		27 Oct	2	0.1	30.8

Table A-8

Analysis of Variance

Term	Sum of Sq	D F	Mean Sq	F
D=D - depth	3482.3	2	1741.15	1.21
L=L - Level of nitrapyrin concentration	3226.87	2	1613.43	1.44
T=T - Temperature	34429.6	2	17214.8	15.37**
S=S - Soil	180.352	1	180.352	0.12
W=W - Week	2471.9	6	411.984	2.93*
DL=DL	1517.87	4	379.468	0.87
DT=DT	5193.76	4	1298.44	0.89
LT=LT	4479.03	4	1119.76	7.97**
DS=DS	2464.94	2	1232.47	0.85
LS=LS	1504.26	2	752.128	1.72
TS=TS	502.685	2	251.343	0.17
DW=DW	1445.61	12	120.468	0.60
LW=LW	752.728	12	62.7273	0.48
TW=TW	8365.89	12	697.158	4.96**
SW=SW	244.71	6	40.785	0.20
DLT=DLT	4183.21	8	522.902	1.2
DLS=DLS	2988.7	4	747.175	1.70
DTS=DTS	5776.99	4	1444.25	5.55**
LTS=LTS	2285.83	4	571.458	1.30
DLW=DLW	1150.78	24	47.9492	0.62
DTW=DTW	1412.71	24	58.8629	0.29
LTW=LTW	3370.05	24	140.419	1.80*
DSW=DSW	3326.23	12	277.186	1.38
LSW=LSW	1357.42	12	113.119	0.56
TSW=TSW	595.1	12	49.5917	0.25
DLTS=DLTS	3504.94	8	438.118	5.63**
DLTW=DLTW	2722.59	48	56.7206	0.73
DLSW=DLSW	771.589	24	32.1495	0.41
DTSW=DTSW	4815.23	24	200.635	2.57**
LTSW=LTSW	3016.72	24	125.697	1.62
DLTSW=DLTSW	3735.12	48	77.815	
TOTAL (CORRECTED FOR MEAN)	115276.	377		

* significant value
** highly significant value

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